ONR FINAL REPORT

GRANT NUMBER: N00014-93-1-1403

PRINCIPAL INVESTIGATOR: Dr. William J. Lennarz

INSTITUTION: State University of New York at Stony Brook

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GRANT TITLE: Isolation and Characterization of Spicule Matrix Protein

REPORTING PERIOD: Final

AWARD PERIOD: 09/01/93 - 08/31/96

I. Studies on the Role of sea Urchin Bone Morphogenetic Protein (suBMP-1) in the Differentiation of Spicule Forming Primary Mesenchymal Cells

Ongoing work in our laboratory has shown that suBMP, a bone morphogenetic protein-1 family member, is the homolog of the *Drosophila* embryonic patterning gene Tolloid. Resequencing the 3' end of the suBMP-1 cDNAs has revealed an extended open reading frame with high homology to human and murine tolloid homologs. Recent studies in the laboratories of Darwin Prockop, Daniel Greenspan, and Effrat Kessler have demonstrated that both human BMP-1 and human Tolloid act as procollagen C-terminal proteinases, releasing the C-terminal propeptide from triple helical procollagen. This proteolytic processing event is necessary for the deposition of collagen fibrils. We have been able to demonstrate the presence of a procollagen C-terminal proteinase (PCP) activity in *S. purpuratus* extracts containing suBMP-1. This PCP activity is heat labile, and demonstrates both time and concentration dependent cleavage. We are currently working to express suBMP-1 as a recombinant protein in mammalian cell culture and to determine if suBMP-1 has PCP activity.

Previous studies in our lab and others have demonstrated that appropriate collagen processing is necessary for gastrulation and spiculogenesis to occur in the developing sea urchin embryo, as well as for calcium carbonate deposition into growing spicules in primary mesenchyme cell culture. Disruption of collagen hydroxylation or crosslinking blocks spicule growth. Removal of the C-terminal propeptide of triple helical procollagen renders the collagen insoluble, allowing it to be deposited into growing collagen fibrils. SuBMP-1 may also play an important role in this process. If suBMP-1 is responsible for the observed PCP activity in *S. purpuratus*, then its function will be essential for collagen deposition and therefore sea urchin development.

Recently, the Tolloid family of metalloproteinases have also been shown to play a key role in the Bone Morphogenetic Protein-4 pathway. The laboratories of Leslie Dale, Eddy De Robertis, Uwe Strahle, and Michael O'Connor have shown that Tolloid and Tolloid homologs cleave and inactivate the Chordin/Short Gastrulation protein, a BMP-4 antagonist. In *Xenopus*, BMP-4 is responsible for the induction of ventral cell fates. Chordin antagonizes the activity of BMP-4 by blocking BMP-4 binding to the BMP-4 receptor. Injection of Xolloid, the *Xenopus* Tolloid homolog, mRNA into the organizer region of the frog embryo results in expanded ventrally fated tissues, and a reduction on dorsally fated tissues by proteolytically inactivating chordin. Xolloid also blocks chordin derived secondary axis formation. Preliminary experiments have shown suBMP-1 to be a functional ortholog of Xolloid in the frog embryo. Experiments are currently underway to determine if suBMP-1 functions in the BMP-4 pathway in the sea urchin. These observations suggest that in addition

to its potential function in collagen processing, suBMP-1 may also function as a morphogen in the BMP-4/Chordin pathway in the sea urchin.

II. Publications Supported by the ONR Grant:

1. Cho, J. W., Partin, J. S. and Lennarz, W. J. (1996) A Technique for Detecting Matrix Proteins in the Crystalline Spicule of the Sea Urchin Embryo. *Proc. Natl. Acad. Sci., USA* 93, 1281-1286.

2. Brown, M. F., Partin, J. S., Killian, C. E. and Lennarz, W. J. (1995) Spiculogenesis in the Sea Urchin Embryo: Studies on the SM30 Spicule Matrix Protein. *Develop. Growth and Differ.* 37, 69-78.

3. Lennarz, W. J. (1994) Fertilization in Sea Urchins: How many Different Molecules are Involved in Gamete Interaction and Fusion. *Zygote* 2, 1-4.

4. Hwang, S.-P. L., Partin, J. S. and Lennarz, W. J. (1994) Characterization of a Homolog of Human Bone Morphogenetic Protein 1 in the Embryo of the Sea Urchin, *S. purpuratus*. *Development* 120, 559-568.

5. Hwang, S.-P. L. and Lennarz, W. J. (1993) Studies on the Cellular Pathway Involved in Assembly of the Embryonic Sea Urchin Spicule. *Exp. Cell Res.* 205, 383-387.

The Research Foundation
of State University of New York

State University of New York at Stony Brook

August 31, 1998

Scientific Officer:
Constance Oliver, Code 341
Office of Naval Research
Ballston Tower Once
800 North Quincy Street
Arlington, VA 22217-5660

RE: N00014-93-1-1403

Dear Dr. Oliver:

Enclosed are three copies of William Lennarz's final technical report for the above-referenced AASERT grant. Also enclosed is a copy of the AASERT reporting form A2-2, the original of which was submitted to the ONR Regional Office in Boston in September of 1996.

If you require any additional information, please contact me at 516-632-9347.

Sincerely,

Kristin Hilbert

Kristin Hilbert
Special Projects Coordinator

cc: ONR Code: 11SP (1)
Attn: AASERT/92
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5660
-Director, Naval Research Laboratory (1)
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FORM A2-2

AUGMENTATION AWARDS FOR SCIENCE & ENGINEERING RESEARCH TRAINING (AASERT) REPORTING FORM

The Department of Defense (DOD) requires certain information to evaluate the effectiveness of the AASERT program. By accepting this Grant Modification, which bestows the AASERT funds, the Grantee agrees to provide the information requested below to the Government's technical point of contact by each annual anniversary of the AASERT award date.

1. Grantee identification data: (R & T and Grant numbers found on Page 1 of Grant)

a. The Research Foundation of State University of New York
University Name

b. N00014-93-11403 (ONR)
Grant Number

c. 4101-141
R & T Number

d. William J. Lennarz
P.I. Name

e. From: 08/31/95 To: 08/31/96
AASERT Reporting Period

NOTE: Grant to which AASERT award is attached is referred to hereafter as "Parent Agreement."

2. Total funding of the Parent Agreement and the number of full-time equivalent graduate students (FTEGS) supported by the Parent Agreement during the 12-month period prior to the AASERT award date.

a. Funding: $ 300,000

b. Number FTEGS: 0

3. Total funding of the Parent Agreement and the number of FTEGS supported by the Parent Agreement during the current 12-month reporting period.

a. Funding: $ 414,800

b. Number FTEGS: 1

4. Total AASERT funding and the number of FTEGS and undergraduate students (UGS) supported by AASERT funds during the current 12-month reporting period.

a. Funding: $ 26,030

b. Number FTEGS: 1

c. Number UGS: 0

VERIFICATION STATEMENT: I hereby verify that all students supported by the AASERT award are U.S. citizens.

Principal Investigator

7/18/96
Date

*Copy-original sent to ONR Regional Office, Boston, on 9/6/96